The function of the Mediator complex in plant immunity

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Upon pathogen infection, plants undergo dramatic transcriptome reprogramming to shift from normal growth and development to immune response. During this rapid process, the multiprotein Mediator complex has been recognized as an important player to fine-tune gene-specific and pathway-specific transcriptional reprogramming by acting as an adaptor/coregulator between sequence-specific transcription factor and RNA polymerase II (RNAPII). Here, we review current understanding of the role of five functionally characterized Mediator subunits (MED8, MED15, MED16, MED21 and MED25) in plant immunity. All these Mediator subunits positively regulate resistance against leaf-infecting biotrophic bacteria or necrotrophic fungi. While MED21 appears to regulate defense against fungal pathogens via relaying signals from upstream regulators and chromatin modification to RNAPII, the other four Mediator subunits locate at different positions of the defense network to convey phytohormone signal(s). Fully understanding the role of Mediator in plant immunity needs to characterize more Mediator subunits in both Arabidopsis and other plant species. Identification of interacting proteins of Mediator subunits will further help to reveal their specific regulatory mechanisms in plant immunity.

Unlike animals, plants are sedentary organisms and lack specialized immune cells. To defend against pathogen infection, each plant cell possesses the ability to recognize invading pathogens and to initiate a timely, accurate and effective immune response.[1] Activation of immune response over normal plant growth and development is accomplished by transcriptome reprogramming.[2-4] During this rapid process, transcription (co)factors perceive the signals relayed by signaling molecules and translate into a functional response through recruiting or releasing (either directly or indirectly) RNA polymerase II (RNAPII).[5]

As a conserved multiprotein cofactor of RNAPII, the Mediator complex is at the core of the transcription machinery. Since its original discovery in yeast, subunits of the Mediator complex have been characterized experimentally or in silico in many eukaryotes.[6-9] Depending on the organism, the Mediator complex consists of ~20 to 30 subunits, which form four Mediator subcomplexes: head, middle, tail and the cyclin-dependent kinase module.[10,11] However, Mediator is not a fixed complex. Several isoforms or alternative forms exist in cells, which may allow it to integrate a multitude of regulatory input.[12] In Arabidopsis, 21 conserved and six species-specific Mediator subunits have been identified.[8] Recently, all known yeast/metazoan Mediator subunits have been identified from 16 plant species using in silico approaches.[9] Although primary sequence similarity between Mediator subunits of plants and other eukaryotes is quite low, high degree of secondary structure similarity has been found across kingdoms, demonstrating the conservation of Mediator organization.[8,9,13]

The function of Mediator is to act as a bridge between RNAPII and DNA-binding transcription factors.[13] In addition to promoting basal RNAPII-mediated transcription, Mediator fine-tunes gene-specific and pathway-specific transcriptional reprogramming by interacting with specific activators/repressors together with general transcription factors at the promoter site.[6,7] Thus, Mediator performs both general and specific roles in regulating gene transcription.[14] In some cases, specific Mediator subunits can regulate a wide range of signaling pathways through selectively interacting with specific transcription factors.[15] In addition, accumulating evidence has demonstrated the role of Mediator as a docking site for a wide range of nuclear machineries such as cohesion proteins and chromatin modifiers,[16-18] which further corroborates the role of Mediator in regulating diverse biological processes.

In *Arabidopsis*, several Mediator subunits have been implicated in multiple signaling pathways including development,[19-25] flowering,[22,26-29] non-coding RNA processing,[30,31] regulation of DNA and protein stability,[8] secondary metabolism,[32] and tolerance to freezing and drought.[33,34] Alternatively, different phytohormones and stresses affect the stoichiometric concentration of Mediator subunits by controlling the transcription of their respective genes in both *Arabidopsis* and rice.[9,35]

Recent progress in defining the function of a number of plant Mediator subunits in plant immunity has further demonstrated the important and diverse roles that individual subunits can possess.[13] Here, we discuss the present understanding of five functionally characterized Mediator subunits in plant immunity.

Mediator Complex Subunit25 (MED25/PFT1)

MED25 was originally described as a positive regulator of shade avoidance in *Arabidopsis* and was termed Phytochrome and

Flowering Time1 (PFT1).[8,26] In plant immunity, MED25 is an important component of basal defense,[27] which is required for jasmonate (JA)-dependent defense gene expression and resistance to the leaf-infecting necrotrophic fungal pathogens *Alternaria brassicicola* and *Botrytis cinerea*.[27] Conversely, MED25 confers susceptibility to *Fusarium oxysporum*, a root-infecting hemibiotrophic fungal pathogen, which is thought to use the host JA pathway to promote host senescence and necrosis.[36] Consistent with this idea, *F. oxysporum* resistance in *med25/pft1* is associated with attenuated JA signaling. Interestingly, induction of salicylic acid (SA)-responsive genes is also attenuated in the *med25/pft1* mutant. However, expression of the SA-responsive genes is not decreased in *med25/pft1* plants under basal condition.[27] Furthermore, there is no detectable enhanced susceptibility to the biotrophic bacterial pathogen *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 and biological induction of systemic acquired resistance (SAR) is not significantly altered in *med25/pft1* plants.[37] In contrast, expression of JA-responsive genes is reduced under both basal and JA-treated conditions in the *med25/pft1* mutant.[27] Therefore, MED25 primarily functions as a positive regulator of JA-responsive gene expression.

Recently, 19 transcription factors have been identified to interact with MED25.[15,34,38,39] They belong to a variety of transcription factor families including AP2/ERF, bHLH, MYB, WRKY, bZIP and zinc finger, demonstrating that the *Arabidopsis* MED25 plays regulatory roles in diverse physiological processes including JA-dependent defense response. Transcriptional activation assays showed that Octadecanoid-Responsive *Arabidopsis* Ap2/Efr59 (ORA59)- and ERF1-dependent activation of *Plant Defensin1.2* (*PDF1.2*), a marker gene of JA-dependent defense response, requires a functional MED25.[39] Additionally, during JA signaling, MED25/PFT1/BER6 (Bestatin-Resistant6) physically interacts with the bHLH transcription factor MYC2 and executes a positive effect on MYC2-regulated expression of JA-responsive genes.[15] Taken together, MED25 acts as part of the general transcriptional machinery in activating ORA59-, ERF1- and MYC2-regulated gene expression in JA signaling pathway.

Mediator Complex Subunit21 (MED21)

The role of MED21 in regulating defense against necrotrophic fungal pathogens is identified through screening of HUB1 (Histone Monoubiquitination1) interacting proteins in *Arabidopsis*.[40] HUB1 is a RING E3 ligase functioning in histone H2B monoubiquitination and contributes to resistance against *A. brassicicola* and *B. cinerea* in *Arabidopsis*.[40] Gene expression profiling showed that *MED21* transcripts accumulate in rosette leaves and can be induced by infection with *Erysiphe cichoracearum* and treatment with defense related phytohormones.[40,41] Consistently, the *MED21* RNAi lines exhibit enhanced susceptibility to *A. brassicicola* and *B. cinerea* at a comparable level to the *hub1* mutants, whereas plants overexpressing *MED21* do not significantly differ from the wild type.[40] Furthermore, both *HUB1* and *MED21* are induced by chitin, a fungal pathogen-associated molecular pattern (PAMP), suggesting their involvement in defense signaling. However, chitin-induced expression of

MED21 is independent of HUB1, indicating that their regulatory relationship is not at the transcription level.[40] Since evidence from yeast suggests an association between ubiquitinated histone H2B and actively transcribed genes,[42,43] MED21 may provide a novel link between H2B ubiquitination and RNAPII in transcription of defense genes in plants. However, HUB1 functions independently of pathways leading to the expression of *PR1* (*Pathogenesis-Related Gene1*, a marker gene of SA-dependent defense response) and *PDF1.2*.[40] Thus, the function of MED21 in activation of SA or JA defense pathways warrants further investigation.

Mediator Complex Subunit15 (MED15/NRB4)

Non-Recognition-of-Bth4 (NRB4) was identified in a genetic screen for components involved in SA response. Conventional mapping revealed that NRB4 is an ortholog of MED15 in *Arabidopsis*.[24] Characterization of the *med15/nrb4* mutants in response to SA revealed high similarity between the functions of MED15 and NPR1 (Non-Expresser Of Pathogenesis-Related Gene1), a master regulator controlling multiple immune responses including SAR in *Arabidopsis*.[24] Similar to *npr1* plants, *med15/nrb4* plants are insensitive to benzol(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), an analog of SA with less phytotoxicity, and cannot detoxify SA supplied in the growth medium. Interestingly, the similarity between *med15/nrb4* and *npr1* extend to enhanced disease susceptibility, biological induction of SAR, effector-triggered immune response and nonhost pathogen response. Although SA and BTH treatment induce a small degree of resistance in *med15/nrb4* plants, they do not induce detectable accumulation of *PR1*. Additionally, transgenic plants overexpressing *MED15* have an enhanced SA response in terms of growth and defense response. On the other hand, there is no evidence for a role of MED15 in JA and cytokinin response.[24] Therefore, the effect of MED15 appears to be limited to SA response.

Overexpression of *NPR1* in *med15/nrb4* does not restore the response to SA, MED15 is not required for NPR1 stability and subcellular localization and no protein-protein interaction exists between MED15 and NPR1; therefore, MED15 appears to function independently or downstream of NPR1 in regulating SA response.[24] However, how MED15 regulates transcription of SA-responsive genes and what kind of coordination exists between MED15 and NPR1 are unknown.

Mediator Complex Subunit16 (MED16/SFR6)

Prior to the identification of Sensitive to Freezing6 (SFR6) as MED16, its role in regulating cold acclimation, drought and osmotic-stress tolerance, development, flowering time and circadian clock has been extensively characterized.[33,44-46] Subsequent work revealed its additional roles in controlling both SA- and JA-dependent defense gene expression and tolerance to UV-C irradiation.[47]

Recently, in a genetic screen for mutants insensitive to exogenous NAD+ (*ien*), a mutant allele of *med16/sfr6* was identified in our laboratory.[37] Exogenous NAD(P) is a potent elicitor of plant

immune response, which induces *PR* gene expression and disease resistance.[48] NAD$^+$ treatment-induced *PR1* gene expression is significantly inhibited in the *med16/sfr6* mutant. Induction of *PR* genes is also drastically decreased in the *med16/sfr6* mutant infected by both the avirulent bacterial pathogen *Pst* DC3000/*avrRpt2* and the virulent bacterial pathogen *Pst* DC3000. Accordingly, both *Pst* DC3000/*avrRpt2* and *Pst* DC3000 grow significantly more in *med16/sfr6* than in wild type. MED16 is not a major contributor to SA biosynthesis, yet BTH-induced *PR* gene expression is dramatically decreased in the *med16/sfr6* mutant, suggesting that MED16 functions downstream of SA as a positive regulator. Similarly, the *med16/sfr6* mutation also blocks the induction of several JA/ethylene (ET)-responsive genes and compromises resistance to *A. brassicicola* and *B. cinerea*. Therefore, MED16 is at the convergence point of SA- and JA/ET-dependent defense pathways.[37]

Interestingly, further mutant characterization revealed that MED16 suppresses SAR-negative regulators, promotes SAR-positive regulators and is required for SAR treatment-induced defense gene expression and SA accumulation in systemic leaves. Consistently, biological induction of SAR is completely compromised in *med16/sfr6* mutants. These results demonstrate that MED16, like NPR1, is a key positive regulator of SAR. Importantly, although the *med16/sfr6* mutation does not affect *NPR1* gene expression and NPR1 protein subcellular localization, it dramatically decreases NPR1 protein levels. Thus, MED16 likely modulates NPR1 protein accumulation in regulating SA responsiveness and basal immunity. Take together, Mediator subunits such as MED16 not only perceive signals from specific transcription activators but also may actively regulate the homeostasis of the transcription activators like NPR1.[37] However, whether MED16 physically interacts with NPR1 or its associated TGA factors during biological induction of SAR is current unclear.

Mediator Complex Subunit8 (MED8)

Similar to *med25/pft1*, a mutation in the *MED8* gene confers susceptibility to *A. brassicicola* but resistance to *F. oxysporum* in *Arabidopsis*, suggesting a role for MED8 in JA-dependent defense.[27] The *med8* mutant displays enhanced susceptibility to *Pst* DC3000, but has no significant defects in biological induction of SAR.[37] Although there is only a slight reduction in MeJA-induced *PDF1.2* expression in the *med8* mutant, the effect of *med8* on *PDF1.2* expression is more visible in the *med25/pft1* mutant background, as the double mutant shows a further decrease in *PDF1.2* expression. As expected, the *med8* mutation has an additive effect with the *med25/pft1* mutation on *F. oxysporum* resistance.[27] Thus, MED8 and MED25 may act independently within Mediator to influence plant defense response.

Conclusions and Perspectives

The balance of hormonal crosstalk strongly influences the outcome of plant-pathogen interactions, including establishment of effective systemic immunity. In this highly coordinated process,

SA signaling triggers resistance against biotrophic and hemibiotropic pathogens, while a combination of JA and ET signaling activates resistance against necrotrophic pathogens. It is generally accepted that SA- and JA/ET-dependent signaling pathways antagonize each other. However, synergistic interactions have been described as well.[49] In plant immunity, Mediator subunits perceive the signals activated by different hormones and initiate defense-associated transcriptome reprogramming.[2-5] MED15 is involved in activation of SA-dependent signaling pathway.[24] MED8 and MED25 mainly regulate JA-dependent signaling, but may also contribute to SA-dependent defense.[27,37] MED16 plays dual roles in mediating both SA and JA/ET signaling pathways as a positive regulator.[37] MED21 regulates resistance against fungal pathogens likely via relaying signals from upstream regulators and chromatin modification to RNAPII.[40] All five Mediator subunits serve as positive regulators of defense against leaf-infecting pathogens but their positions in conveying phytohormone signals are different. Identifying their interacting proteins and integrating them into the current plant defense signaling network will help to fully understand the mechanism by which Mediator regulates plant immunity. Indeed, physical interactions between MED25 and a diversity of transcription factors suggest that MED25 acts as an integrative hub within Mediator to regulate a wide range of physiological processes including defense response.[15,39]

Plant Mediator is a macromolecular unit composed of members of 34 subunits.[9] Besides MED8, MED15, MED16, MED21 and MED25, it is unknown whether the remaining subunits function in plant immunity. In *Arabidopsis*, through the analysis of 11 Mediator subunit mutants, MED8 was identified as a regulator of resistance to necrotrophic pathogens.[27] In a recent screen of 64 available T-DNA insertion mutants, no additional Mediator subunit mutants with a measurable phenotype in SA response had been found.[24] However, 18 out of the total 64 T-DNA insertion mutants used in this screen were not homozygous, which may lead to biased conclusion since most Mediator subunit mutations are recessive, not to mention that some mutations may affect other defense pathways. Therefore, a more comprehensive screen may identify additional Mediator subunits functioning in multiple plant defense pathways. Moreover, a wheat homolog of MED25 can complement the defense and the developmental phenotypes of the *Arabidopsis med25/pft1* mutant, indicating the function conservation of MED25 in higher plants.[27] However, whether the function conservation can be extended to other Mediator subunits is unclear and the exact role of Mediator subunits in defense response of other plant species especially crops remain to be addressed in future research.

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